



Product Information

PROTEASE TYPE XIV
from *Streptomyces griseus*
Sigma Prod. No. P5147

CAS NUMBER: 9036-06-0

SYNONYMS: Pronase E, Actinase E

PHYSICAL DESCRIPTION:

Appearance: white to tan powder

KINETIC PARAMETERS:

Pronase E is highly stable in the pH range 5.0 to 9.0, but fairly unstable below pH 4 and above pH 10.¹ The neutral components in the enzyme mixture are stable at pH 5-9 with calcium present; the alkaline components are stable over pH 3-9, with optimal activity at pH 9-10. The aminopeptidase and carboxypeptidase components are stable at pH 5-8, in the presence of calcium ion.² The optimum activity will be at pH 7-8.²

The product can be completely inactivated by heating above 80°C for 15-20 minutes.¹ Some components of the mixture are inactivated more quickly than others. Adding excess EDTA results in irreversible loss of about 70%. The mixture retains activity in 1% SDS (w/v) and 1% Triton (w/v).

INHIBITORS:

No single substance will inhibit all the different proteases in the mixture (see structure). Diisopropylfluorophosphate, PMSF and EDTA have been used with some success.²

STRUCTURE:

This product is a mixture of at least three caseinolytic activities and one aminopeptidase activity. The caseinolytic enzymes were named as *Streptomyces griseus* Protease A, *Streptomyces griseus* Protease B and *Streptomyces griseus* Trypsin.³ The amino acid sequences and molecular weights have been reported: 18,093 for Protease A⁴, 18,629 for Protease B⁵, and 22,918 for *S. griseus* Trypsin.⁶ Properties of this trypsin have also been reported.⁷ Values of 16,000 and 18,000 for two different proteolytic activities have been reported⁸; molecular weights, usually determined by gel filtration, range from 16,000 to 27,000.^{1,2}

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SUBSTRATES AND SPECIFICITY:

"Pronase E" is the name given to a group of proteolytic enzymes produced by *Streptomyces griseus* K-1. At least 10 proteases are in the mixture, five serine-type proteases, two zinc endopeptidases, two zinc leucine aminopeptidases and one zinc carboxypeptidase.² Digestion with the product has been useful when extensive or complete degradation of protein is required. Pronase digestion is particularly useful since tryptophan, serine, threonine, asparagine and glutamine are easily destroyed by the usual acid hydrolysis procedures. This protease mixture is so nonspecific that it can digest casein to the extent of >70% as mono-amino acids.^{1,2} It was shown to be much more effective in digestion of casein than trypsin, chymotrypsin and several other proteases.⁹

METHOD OF PREPARATION:

This mixture of proteases is produced from the culture broth of *Streptomyces griseus* and is isolated chromatographically.¹

STORAGE / STABILITY AS SUPPLIED:

The lyophilized powder is extremely stable if stored frozen and dry. Although Sigma has not verified the claim, customers have reported that retained samples have maintained full activity after fifteen years of proper storage.

SOLUBILITY / SOLUTION STABILITY:

The product dissolves in 0.01 M sodium acetate with 0.005 M calcium acetate at pH 7.5 at 37°C; at 0.2 mg/mL the clear solution ranges from colorless to light tan. Calcium ion is recommended for protection from autolysis. The activity of a dilute enzyme solution containing 0.01 to 0.1 M calcium ion was stable over 24 hours at neutral pH at 2-8°C. Pronase E is stable at 4°C for at least six months. Stock solutions of 5 to 20 mg/mL in water are usually stored at -20°C.²

USAGE:

DNA isolation:²

For Pronase E is usually prepared as a stock solution, and prior to storage at -20°C, the solution is first heated to 56°C for about 15 minutes, then incubated at 37°C for 1 hour. This encourages self-digestion, to eliminate DNase and RNase contamination. The enzyme is added to a DNA sample (in the presence of 0.5-1% SDS to disrupt DNA protein interactions) typically at 250-500 µg protein/mL, then incubated at 37°C for 1-4 hours.

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USAGE: (continued)

For protein hydrolysis, dissolve about 0.2 micromole of protein in 0.2 mL of 50 mM ammonium bicarbonate buffer at pH 8 (or phosphate buffer pH 7). Add pronase to 1% (w/w) and incubate at 37°C for 24 hours. It may be necessary to add aminopeptidase M at 4% (w/w) and incubate at 37°C for another 18 hours.

Additional uses include hydrolysis of amino acid amides¹⁰, pretreatment of liver tissue sections to enhance the intensity of immunostaining¹¹, regeneration of certain types of affinity columns¹², and removal of protein in DNA/RNA isolations.¹³ Detailed references concerning isolation, properties and structure are noted.^{14a-f}

In addition to P5147, Sigma offers cell culture tested P8811 and molecular biology tested P6911.

UNIT DEFINITION:

One unit will hydrolyze casein to produce color equivalent to 1.0 μ mole (181 μ g) of tyrosine per min at pH 7.5 at 37°C (color by Folin-Ciocalteu reagent).

Activity: Approximately 4 units per mg solid. Assay protocol available on Sigma's WWW website or on request from Technical Service.

REFERENCES:

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